REMARKS

Reconsideration and allowance are respectfully requested.

Claims 40-50 are pending. The scope of claims 42 and 46 is not changed because their amendment is directed to clarifying instead of limiting the originally intended scope of protection.

The listing of DE 195257484 was not initialed by the Examiner in the PTO-1449 returned with the Action. Applicants submit that the requirements for an information disclosure statement were satisfied because the Int'l Search Report (ISR) considered by the Examiner satisfies 37 CFR § 1.98(a)(3)(i) and M.P.E.P. § 609.04(a)III because the requirement for a concise explanation of relevance can be satisfied by submitting an English-language version of the search report which indicates the degree of relevance found by the foreign patent office ("This may be an explanation of which portion of the reference is particularly relevant, to which claims it applies, or merely an "X", "Y", or "A" indication on a search report" at 600-154). Here, the ISR previously considered by the Examiner (another copy is attached for convenience) satisfies this requirement. The return of an initialed copy of the PTO-1449 is requested to confirm consideration of DE 195257484. If the Examiner alleges non-compliance with any other requirement of the regulations, additional time under 37 CFR § 1.97(f) is requested to provide the missing content because no reason was given in the Action for not considering the listed document.

The Examiner objected to the Declaration because the correction of an inventor's address was not initiated and dated. In response, an Application Data Sheet in compliance with the regulations is submitted herewith. Entry of same and withdrawal of the objection are requested.

The Examiner also objected to the specification because it lacks a brief description of the drawings. Entry of same by amendment and withdrawal of this objection are requested.

35 U.S.C. 112 – Definiteness

Claim 42 was rejected under Section 112, second paragraph, as being allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants traverse.

The Examiner's suggestion for amending claim 42 to clarify its metes and bounds is gratefully acknowledged. Adoption of her suggestion moots the rejection.

Withdrawal of the Section 112, second paragraph, rejection is requested.

Applicants' Comments on the Claims

With respect to the section of the Office Action entitled "Summary of the claim invention and claims interpretation, the Examiner's consideration of Applicants' following comments is requested.

Applicants were invited on page 4 of the Action to clarify the meaning of the term "a known epitope" if they did not agree with the Examiner's interpretation that all epitopes used to practice the invention are known. The intended purpose of the word "known" in claim 46 was to focus on the situation where a T cell-activating peptide is provided for presentation to T cells in the sample by adding to the sample a peptide epitope. This necessarily requires that a relevant T cell epitope has been pre-identified by conventional epitope mapping techniques (i.e., the epitope added and presented is "known").

It needs to be kept in mind, however, that T cell epitopes are presented to T cells by antigen presenting cells in conjunction with MHC antigen. Hence, the presentation of T cell-activating peptide in (b) may alternatively be achieved by using whole antigen (e.g., a native glycoprotein) or fragments thereof which are then processed by antigen presenting cells (APC) in the sample to provide the T cell-activating peptide. In such an alternative, it is not necessary to know (or pre-identify) the identity of the epitope that is being presented to the T cell. It suffices for one of ordinary skill in the art to know that one or more relevant T cell epitopes will be generated and presented on the APC to T cells in the sample. But the identity of the T cell-activating peptide would not necessarily

be known because only the identity of the precursor (i.e., whole antigen or fragments thereof) is needed prior to practicing the invention.

Therefore, to clarify known versus unknown epitopes, the term challenged by the examiner is replaced by --pre-identified--.

It was alleged on page 5 that "monitoring the progress or effect of an infection or vaccine" is an intended use of the invention instead of a claim limitation. In response, it is noted that claim 40 originally recited the positive limitation that the method is "applied to diagnosis or monitoring of infection with an intracellular pathogen." This limitation should be weighed in considering the patentability of the claimed invention because it is clearly different form simply specifying that a method is suitable for diagnostic use. To ensure such consideration, claim 40 is amended to clarify that diagnosis or monitoring is achieved by addition of (e) comparing the number of T cells detected to background or control numbers to determine diagnosis or monitoring of the pathogenic infection.

By way of introduction, the subject invention concerns a method of assaying the frequency of *ex vivo* effector T cells in a T cell-containing sample such as a plasma sample in order to establish the presence or absence of an intracellular pathogenic agent as defined by the specific peptides which are selected to stimulate the T cell receptor on 'target' effector T cells. The 'effector T cell' is a discrete type of T cell as shown by its ability to release interferon gamma in response to T cell-activating peptide without needing to divide or differentiate during prior *in vitro* culture, and the realization that it might represent a 'dynamic' marker in blood for the presence or absence of a specific disease was essential to development of the invention.

Conventional definitions in the immunological art for the various T cell types involved in antigen-specific cytokine responses are as follows:

> Naïve T Cells are cells that have never encountered a given antigen (such as that derived from a pathogen) and need to be activated, and then to proliferate and differentiate, before being able to secrete cytokines in response to that antigen. The process of activation involves the 'priming' of T cells with the so-called cognate antigen in the presence of co-stimulatory molecules (e.g., CTLA-4).

Memory T Cells are cells that have already encountered cognate antigen, and activate more rapidly/easily than naïve T cells on re-encountering that antigen, before proliferating, differentiating, and ultimately secreting cytokines.

Effector T Cells (referred to in some textbooks as 'armed effector cells') have already encountered cognate antigen, and are already active *in vivo*, i.e., they are in a state of being able to secrete cytokines in response to specific antigenic protein or peptide molecules without prior proliferation or differentiation. Both memory and effector T cells are 'antigen experienced' but, in comparison with effector T cells, memory T cells are both quiescent and long lived.

The aim of the inventors is to establish a simple and reliable test for detecting the presence or absence of an intracellular pathogen in a host. The state of the art in 1996 (e.g., Ahmed & Gray, Science 272:54-60, 1996; see entry at top of sheet 3 of the IDS) included the realization that effector T cells are maintained only in the presence of foreign antigen, whereas memory T cells are maintained even when foreign antigen is lost from the host. Applicants, however, arrived at their invention by appreciating that detection of pathogen-specific effector T cells exclusively *ex vivo* is an achievable objective, and that such cells can act as a 'dynamic' marker for the existence of an intracellular pathogen in a human host. The various practical steps which needed to be assessed and/or comprehended in order to successfully achieve this unique goal are set out below. A concept (see Concept 6 listed below) which led to making the invention was the realization that pathogen-specific effector T cells can be detected by an ELISPOT assay, above background levels, after an incubation time which is too short (e.g., as quickly as six hours) for 'interference' by memory cell progeny. This realization only came to the Applicants from their studies of influenza infection - studies that were reported in Lalvani et al. J. Exp. Med. 186:859-865 (1997) after the effective filing date of this application.

Concept 1: The presence of antigen is essential to maintain population of circulating activated effector T cells *in vivo*.

Concept 2: Effector T cells respond to antigen *in vitro* without need to proliferate or differentiate.

Concept 3: Memory T cells only respond *in vitro* following proliferation (generally after 24 hours of *in vitro* stimulation with antigen).

Concept 4: In view of Concepts 1-3 above, selective detection of pathogen-specific effector T cells could provide a dynamic surrogate marker for the presence of, or recent infection by, pathogen in a host.

Concept 5: ELISPOT is a sensitive method, and able to enumerate responsive peptide-specific T cells *in vitro*.

Concept 6: ELISPOT is also sufficiently sensitive to detect effector T cells above background levels before any interference from memory progeny cells occurs at later incubation times.

Concept 7: In view of anticipated short *in vitro* incubation times, fresh cells should be introduced directly into ELISPOT wells.

Typically, a human cell culture *in vitro* divides every 24 hours - see the statement on the first page, first two lines, under the section entitled "Phases of the Cell Cycle" in Cooper, *The Eukaryotic Cell Cycle* (second entry on final sheet of the IDS). This observation substantiates using an incubation time of 4-24 hours (as set out in Claim 47) as a guide that will ensure that any T cell types that need to divide before responding to the T cell-activating peptide will not be detected. Accordingly, a memory T cell will take about 24 hours to divide (not including a finite but relatively short *in vitro* activation time), whereas a quiescent T cell such as a naïve T cell will be expected to take even longer to activate before the transition to the proliferation and differentiation step.

Therefore, any teaching of assay protocols which incubate T cells with foreign antigen for more than 24 hours will significantly increase the risk that both effector and memory/naïve T cells will be activated to produce gamma interferon. And if memory cell activity contributes to the final enumeration of T cell frequency, the diagnostic power of the assay would have been compromised because, unlike effector T cells, memory T cells are not a dynamic marker. Thus, Applicants submit that no ELISPOT protocol requiring an incubation time in excess of 24 hours can be automatically considered to anticipate the subject invention because of complications arising from significant numbers of memory and naïve T cells dividing and differentiating.

35 U.S.C. 102 – Novelty

A claim is anticipated only if each and every limitation as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is claimed. See *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

Claims 40-41, 43 and 46-50 were rejected under Section 102(b) as allegedly anticipated by Hagiwara et al. (AIDS Res. Hum. Retrovir. 12:127-133, 1996). Applicants traverse. This document was cited as "Hagiwara" in the Action.

Hagiwara describes experiments which measure the response of T cells to the plant lectin phytohemagglutinin (PHA), which is a mitogen. No attempt was made in Hagiwara to introduce antigens (or fragments thereof) *in vitro* to which isolated human T cells might have been previously 'primed' *in vivo.* PHA is also not an antigen associated with infection by an intracellular pathogen.

It is well known that lymphocytes can be activated by PHA, which has a marked selectivity for T cells as compared to B cells or other mononuclear cells. But the plasma membrane binding sites for PHA appear to be distinct from those for peptides/proteins derived from foreign antigens on T-lymphocytes. More recent work suggests that PHA nonspecifically cross-links human T cell surface membrane glycoproteins, including the T cell receptor and possibly the CD3 molecule, thereby inducing polyclonal activation (i.e., activation of a wide spectrum of 'clonal' T cells irrespective of their cognate antigen specificity) and agglutination of T cells (Abbas et al., *Cellular and Molecular Immunology*, pg. 166, 2005; a copy of which is attached). The binding of this mitogen to numerous sites on the T cell surface may effect sufficient membrane perturbation to induce a calcium influx, and thereby the generation of diacylglycerols (see Valentine et al., Eur. J Immunol. 15:851-854, 1985; a copy of which is attached) which in turn activate gene expression. To summarize, what is known about PHA is such that Applicants submit that a person skilled in the immunological arts would be very unlikely to describe or

consider any aspect of the nature of the binding of PHA to the T cell surface as being '*peptide-specific*' or to affect '*peptide-specific effector T cells*'.

In contrast, the process of activation of naïve T cells in response to proteinaceous antigens, and their subsequent proliferation and differentiation into effector T cells – a process which represents one of the primary cell-mediated immune responses against a variety of foreign antigens *in vivo* – is highly specific and very well characterized.

Applicants teach that their application is "concerned with a method for activating peptide-specific T cells" (see page 1, lines 5-6, of the specification) and, later with "a method of assaying for peptide-specific effector T-cells" (see page 2, lines 7-8, of the specification). The skilled immunologist knows that these statements refer to molecular mechanisms involved in the primary cell-mediated immune response referred to above. Mechanistically, a given effector T cell which is primed for activation (as a result of an earlier maturation process during which the cell develops a capability to recognize its cognate antigen) will in general bear a homogeneous set of T cell antigen receptors which will only recognize one (or possibly a few) specific antigenic (or immunogenic) peptide fragments. As already noted above in relation to claim 46, these peptide fragments (T cell epitopes) are 'presented' to effector T cells via molecules encoded by a group of genes termed 'the Major Histocompatibility Complex' (MHC) such as, for example, human HLA proteins, with which such fragments are associated prior to encountering the T cell. Within a given population of T cells in human blood, only a small percentage of them will respond to (i.e., be activated by) the specific 'presented' protein or peptide fragment. PHA is not a T cell-activating peptide.

This contrasts dramatically with the effect of PHA, in which virtually all T cells in a given population will be activated through a nonspecific cross-linking mechanism. In fact, PHA is often used as a 'control' to ensure that a given preparation of isolated T cells is 'viable' before a more definitive experiment is conducted on them. Indeed, this is the purpose of its use in the studies reported in Hagiwara.

Claim 40 defines the scope of the invention as "a method of assay in which peptide-specific effector T cells are enumerated." Applicants submit that a skilled

immunologist knows that this definition unambiguously excludes any assay method which might measure the effects of PHA, or other similar mitogens which act nonspecifically, via detecting cytokine production.

In summary, Applicants submit that the claimed invention is not anticipated by Hagiwara since the immunological response being measured in the cited document using PHA does not reflect the ability of the T cell population to launch a primary immune response to a pathogen-specific antigen.

Claims 40, 43, 46-47 and 49-50 were rejected under Section 102(b) as allegedly anticipated by Klinman et al. (Curr. Protocols Immunol. 6.19.1-6.19.8, 1994). Applicants traverse. This document was cited as "Klinman" in the Action.

Klinman is at best ambiguous as regards its teaching of how to activate T cells with antigens *in vitro*. It was noted on page 7 of the Action that "Klinman discloses that for standard ELIspot analysis, the stimuli can be added directly to the cells while in the nitrocellulose-backed microtitre plates." But omitted after the quote is the sentence that follows immediately therafter, "For information about stimulating cells, see UNITS 3.12, 3.13, 7.10 & 7.19." Taking the latter into account, Applicants submit that Section 7 of the ELISPOT protocol in Klinman discloses that T cells should be stimulated with antigen for 2-6 days. The units of particular relevance for T cells, as cited in the Section 7 'discussion' section, would appear to be Units 3.12 and 7.10 (copies of which are provided). Those units suggest stimulation with antigen for a period of several days. Furthermore, one also needs to look at the precise wording of Section 7 with care. It refers to stimulation of cells for the assay more precisely as follows: "*Stimulation can occur prior to addition of cells* (note: this can only sensibly mean prior to addition of cells to wells of the plate) *or during incubation on the plate.*" There is then citation to the other units noted above for information on stimulation of T cells. Hence, the only way to reconcile the time period for the protocol in Section 8 with the preceding Section 7 is to conclude that Klinman intended separate consideration to be given to stimulation of T cells and actual detection of cytokine release with reference to the chosen cytokine for detection.

In summary, Applicants submit that it is only by additionally referring to Units 3.12 and 7.10 in conjunction with Steps 7 and 8 as outlined in Klinman that one can begin to appreciate correctly the nature of the protocols being proposed. Consequently, Klinman does not anticipate the claimed invention because the cited document discloses the need to incubate T cells for several days, a period which will result in memory T cells being activated along with effector T cells. This clearly fails to teach a time period "to permit interferon-γ release by only those T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide and are capable of immediate effector function without the need to effect division/differentiation by *in vitro* culture in the presence of the T cell-activating peptide" as required by Applicants' invention and teaches away therefrom.

Withdrawal of the Section 102 rejections is requested because all limitations of the claimed invention are not disclosed by the cited documents.

35 U.S.C. 103 – Nonobviousness

To establish a case of prima facie obviousness, all of the claim limitations must be taught or suggested by the prior art. See M.P.E.P. § 2143.03. Obviousness can only be established by combining or modifying the prior art teachings to produce the claimed invention if there is some teaching, suggestion, or motivation to do so found in either the references themselves or in the knowledge generally available to a person of ordinary skill in the art. See, e.g., *In re Fine*, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988); *In re Jones*, 21 USPQ2d 1941, 1943-44 (Fed. Cir. 1992).

It is also well established that the mere fact that references can be combined does not render the resultant combination obvious unless the desirability of that combination is also taught or suggested by the prior art. See *In re Mills*, 16 USPQ2d 1430, 1432 (Fed. Cir. 1990). Therefore, even if all elements of the claimed invention were known, this is not sufficient by itself to establish a prima facie case of obviousness without some evidence that one would have been motivated to combine those teachings as proposed by the Examiner. See *Ex parte Levengood*, 28 USPQ2d 1300, 1302 (B.P.A.I. 1993). Finally, a determination of prima facie obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

Applicants stress that their invention could not have been conceived until all of the Concepts 1-6 as outlined above had been understood and/or realized.

Claims 40 and 45 were rejected under Section 103(a) as allegedly unpatentable over Hagiwara et al. (AIDS Res. Hum. Retrovir. 12:127-133, 1996). Applicants traverse.

Applicants submit that the cited document alone does not teach or suggest the claimed invention, which depends on peptide specific responses of T cells via the T cell receptor, a mechanism far removed from that by which PHA acts nonspecifically on T cells because PHA is not a T cell-activating peptide. Furthermore, Applicants' invention requires that the enumeration of peptide-specific effector T cells be used to indirectly detect the presence or absence of intracellular pathogen in a host. Hagiwara fails to teach or suggest this limitation, or even a single one of Concepts 1-6 (see above).

Claims 40, 43-44 and 46-48 were rejected under Section 103(a) as allegedly unpatentable over Miyahara et al. (J. Immunol. Meth. 181:45-54, 1995) in view of Hagiwara et al. (AIDS Res. Hum. Retrovir. 12:127-133, 1996). Applicants traverse.

Firstly, Applicants strongly disagree that one of ordinary skill in the art would have been motivated to combine the teachings of Hagiwara and Miyahira. The former document does not teach use of cultured cells and indeed, as noted on page 8 of the Action, "Hagiwara teaches that ELIspot results are divergent when studying PBMC that had been cultured and stimulated *in vitro*" and "Hagiwara teaches that since the type and amount of cytokine produced *in vitro* can be altered by the culture conditions employed, inconsistent results from such studies are not unexpected." Miyahira actually endorsed the use of such 'culture conditions' by describing culturing and stimulating T cells *in vitro*. Thus, Miyahira teaches precisely what Hagiwara advises against.

Secondly, if the *in vitro* response of T cells to specific peptides were to be examined, an essential requirement is that peptides capable of binding to T cell receptors must be placed in proximity to T cells for some finite time period. One of ordinary skill in the art would have concluded that Hagiwara provides absolutely no useful teaching in this regard since PHA binds via a different mechanism, which was characterized well before 1996, and would therefore be expected to stimulate these cells with different characteristics and kinetics compared with the more specific responses triggered via the

T cell receptor. In fact, the accepted wisdom in 1996 was that long *in vitro* incubation times are needed in order to obtain T cell responses to specific peptides derived from cognate antigen.

In the light of both of the above, it is strongly suggested that one of ordinary skill in the art would NOT have been motivated in any way to combine the two cited documents in the hope of obtaining an improved result because their objectives and results would not have been seen as mutually complementary.

Furthermore, in a statement at page 52, end of the first paragraph in the right-hand column, of Miyahira, the validity of ELISPOT assays for quantitative use is argued on the ground that they generate very similar results to those obtained with standard Limiting Dilution Analysis (LDA). It was well known in the art that LDA makes no distinction between memory progeny T cells and effector T cells, and this teaching of Miyahara therefore also teaches away from Applicants' invention. By way of contrast, Applicants' invention depends crucially on the premise that it is NOT equivalent to LDA.

Claims 40-43 and 46-48 were rejected under Section 103(a) as allegedly unpatentable over Surcel et al. (Immunol. 81:171-176, 1994) in view of Sørensen et al. (Infect. Immun. 63:1710-1717, 1995). Applicants traverse.

Applicants dispute that one of ordinary skill in the art would have been motivated to combine Surcel and Sørensen in the manner proposed in the Action because such an assertion fails to appreciate significant aspects Surcel as discussed below.

Firstly, Surcel specifies that the method for gamma interferon assays requires use of non-fresh cells (note that a preincubation time of 72 hours is specified for before transfer of PBMCs to antibody-coated plates). Such a protocol is not designed with a view to measuring responses of only *ex vivo* effector T cells but will additionally detect memory progeny cells.

Specifically, the 'Material and Methods' section of Surcel sets out two different ELISPOT assays, one using detection of the cytokine IL-4 and another using detection of interferon-γ. In the case of IL-4 detection, freshly isolated PBMCs are used with an incubation time of 20 hours. Importantly, however, Surcel additionally explicitly directs that when detecting interferon-γ, freshly-isolated PBMCs should be preincubated in

culture medium for 72 hours prior to transfer to the anti-interferon-γ antibody-coated plates. Such incubation can only sensibly be with antigen. Thus, the clear teaching in Surcel is of the use of non-fresh T cells being applied to antibody-coated plates for the application of the ELISPOT technique (see, in particular, page 172, first six lines of the third paragraph in the right-hand column, under the heading "Cytokine-producing cells").

Whether cells used in gamma interferon assays are further incubated for 20 hours on a surface carrying an immobilized antibody to gamma interferon is a moot point, in view of the ambiguous nature of the technical description. Notwithstanding this ambiguity, however, the interferon-γ detection assay of Surcel does not provide "a fluid containing fresh T cells, which have not been cultured *in vitro,* in contact with a surface carrying an immobilied antibody to interferon-γ" in claim 40(a), which is a requirement of the claimed invention.

Applicants' invention may be used to provide a convenient single-stage plate-based method for detection of *ex vivo* effector T cells suitable for diagnosing or monitoring infection with an intracellular pathogen. Apart from Concept 5, there is no other teaching in Surcel which would render Applicants' invention obvious. Surcel clearly shows that the author were not in possession of the other concepts underlying Applicants' invention, and, if anything, teaches one of ordinary skill in the art away from the claimed invention by measuring effector and memory T cells simultaneously. Indeed, Surcel suggests in the final nine lines of the second paragraph in the 'Discussion' section, further investigation of lengthy antigen stimulation on the order of days!

As noted on page 10 of the Action, Sørensen discloses the value of ESAT-6 in eliciting release of gamma-interferon from T cells. But if ESAT-6 protein and/or peptide fragment thereof were used as the 'activating antigen' in Surcel's ELISPOT method, the claimed invention would not be the result, nor would there be any suggestion of the claimed invention. In any event, Surcel already discloses the use of mycobacterial antigens in ELISPOT assays (see, for example Figure 2), and although these antigens are crude, they do generate T cell responses *in vitro*. Thus, Sørensen adds nothing to Surcel which enables one of ordinary skill in the art to better grasp the various concepts underlying Applicants' invention.

Withdrawal of the Section 103 rejections is requested because the invention as claimed would not have been obvious to one of ordinary skill in the art at the time it was made.

Conclusion

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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